Successful Adenovirus-Mediated Wild-Typ p53 Gene Transf r in Patients With Bladd r Cancer by Intravesical Vector Instillation

By Jürgen Kuball, Shu Fen Wen, Joachim Leissner, Der k Atkins, Patricia Meinhardt, Erlinda Quijano, Heidrun Engler, Beth Hutchins, Daniel C. Maneval, Michael J. Grace, Mary Ann Fritz, Stefan Störkel, Joachim W. Thüroff, Christoph Huber, and Martin Schuler

Purpose: **To study safety, feasibility, and biologic activity of adenovirus-mediated *p53* gene transfer in patients with bladder cancer.**

Patients and Methods: **Twelve patients with histologically confirmed bladder cancer scheduled for cystectomy were treated on day 1 with a single intratumoral injection of SCH 58500 (rAd/p53) at cystoscopy at one dose level (7.5×10^{11} particles) or a single intravesical instillation of SCH 58500 with a transduction-enhancing agent (Big CHAP) at three dose levels (7.5×10^{11} to 7.5×10^{13} particles). Cystectomies were performed in 11 patients on day 3, and transgene expression, vector distribution, and biologic markers of transgene activity were assessed by molecular and immunohistochemical methods in tumors and normal bladder samples.**

Results: **Specific transgene expression was detected in tissues from seven of eight assessable patients treated with intravesical instillation of SCH 58500 but in none of three assessable patients treated with intratumoral injection of SCH 58500. Induction of RNA and protein expression of the p53 target gene *p21/WAF1* was demonstrated in samples from patients treated with SCH 58500 instillation at higher dose levels. Distribution studies after intravesical instillation of SCH 58500 revealed both high transduction efficacy and vector penetration throughout the whole urothelium and into submucosal tumor cells. No dose-limiting toxicity was observed, and side effects were local and of transient nature.**

Conclusion: **Intravesical instillation of SCH 58500 combined with a transduction-enhancing agent is safe, feasible, and biologically active in patients with bladder cancer. Studies to evaluate the clinical efficacy of this treatment in patients with localized high-risk bladder cancer are warranted.**

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AN ESTIMATED 261,000 new cases of bladder cancer are diagnosed worldwide per year. Bladder cancer is prevalent in the developed countries, where it affects mainly men and is frequently associated with a history of tobacco smoking or some occupational exposures, and in Northern Africa and Western Asia, where it is related to endemic infection with the parasite *Schistosoma mansonii*.[1] In the Western world, 70% to 80% of patients present with superficial bladder tumors, which can be treated with transurethral resection.[2,3] However, patients with less differentiated, large or multilocular bladder tumors as well as patients with carcinoma in situ or stage I bladder cancer are at high risk for tumor recurrence and development of muscle-invasive disease or distant metastases.[4,5] Treatment strategies for such high-risk patients include local resection with close surveillance,[2] local resection and intravesical therapy using bacillus Calmette-Guerin or cytotoxic agents,[6-8] or radical cystectomy with urinary diversion or reconstructive surgery.[9,10] Radical cystectomy provides optimal control of the bladder tumor, but at the price of organ loss. Intravesical and systemic medical therapies have substantial toxicities and bear the risk of local recurrence or tumor progression. Thus, new bladder-preserving treatment options for high-risk bladder cancer are required.

Mutations of the *p53* tumor suppressor gene are the most common genetic alteration in human cancers.[11] The role of p53 in the prevention of oncogenic transformation, maintenance of genetic stability, and sensitivity to commonly used cancer treatments is well established.[12,13] In some but not all studies, nuclear accumulation of p53 as an indicator for mutations in the p53 DNA binding domain was associated with an adverse prognosis in patients with bladder cancer.[14-17] Hence, somatic gene transfer of the *p53* tumor suppressor is an attractive new treatment modality for malignant bladder tumors. Preclinical cancer models have

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From the Departments of Medicine III and Urology, Johannes Gutenberg University, Mainz, and Department of Pathology, University Herdecke Witten, Germany; Canji Incorporated, San Diego, CA; and Schering-Plough Research Institute, Kenilworth, NJ.

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Address reprint requests to Martin Schuler, MD, Department of Medicine III, Johannes Gutenberg University, D-55101 Mainz, Germany; email: m.schuler@3-med.klinik.uni-mainz.de.

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demonstrated that the expression of *p53* by viral or nonviral gene transfer technology effectively induced apoptosis or sensitized cancer cells to drug- or radiation-induced cell death.[18] These results hav fostered th translation of *p53* gen therapy into early clinical studies, which were conducted in patients with advanced lung, head and neck, ovarian, or liver cancers.[19-23] Using intratumoral injection of adenoviral[20-22,24] or retroviral[19] *p53* expression vectors, local transgene expression[20,22] and evidence for local tumor regressions and induction of apoptosis[19,24] were reported from several phase I and pilot studies. However, the only controlled phase II study in patients with newly diagnosed advanced non–small-cell lung cancer (NSCLC) failed to demonstrate a significant clinical benefit from local *p53* gene transfer by intratumoral vector injection in combination with an effective first-line chemotherapy.[25] One reason for this apparent clinical inactivity might be insufficient gene delivery and transduction after intratumoral injection of adenoviral *p53* expression vectors. Systematic studies of these important parameters, however, are absent in cancer patients.

One way to overcome the potential limitations of the intratumoral injection approach is the instillation of high-vector doses into cavitary organs, such as the pleural space,[26] peritoneal cavity, or bladder. This should allow a homogeneous vector distribution along the tumor surfaces, as opposed to a vector distribution along the track of an injection needle. Preclinical studies have demonstrated the feasibility of this approach and have highlighted the importance of the addition of transduction-enhancing agents to maximize transgene expression in the bladder.[27,28]

To address this hypothesis, a study of safety, feasibility, and biologic activity of an intravesical instillation or an intratumoral injection of an adenoviral expression vector encoding wild-type p53 (SCH 58500) was conducted in patients with invasive bladder cancer. To allow assessment of vector distribution, transgene expression, and induction of p53 target genes or additional markers of biologic activity after the study treatment, only patients scheduled for radical cystectomy were enrolled onto this trial, enabling extensive tissue sampling for these analyses.

PATIENTS AND METHODS

Patients

Adult patients with histologically confirmed, muscle-invasive bladder cancer and indication for radical cystectomy were eligible for enrollment. Additional inclusion criteria were a life expectancy of at least 3 months, a Karnofsky performance score of at least 70%, and the absence of any clinical or laboratory evidence (WBC count ≥ 3,000/μL, absolute neutrophil count ≥ 1,000/μL, platelet count ≥ 100,000/μL, creatinine < 1.5 mg/dL, bilirubin < 1.5 mg/dL, AST and ALT < 1.5 times the upper limit of normal, and prothrombin and partial thromboplastin times within normal limits) for dysfunction of the hematopoietic, liver, renal, or coagulation systems. An interval of at least 4 weeks between prior chemotherapy, radiation, or major surgery was mandatory. Pregnant or nursing women, fertile women not practicing medically accepted contraception, patients with uncontrolled serious bacterial, fungal, or viral infections, human immunodeficiency virus–positive patients, and immunosuppressed patients were not eligible. Molecular or immunohistochemical evidence for an intratumoral *p53* mutation was not required for eligibility. All patients provided written informed consent. After written informed consent, control tissue samples were obtained from patients with advanced bladder cancer or patients with nonmalignant bladder disease treated by cystectomy.

Study Design

This was an open-label, single-center, phase I dose-escalation study of a single intratumoral injection (part A) or a single intravesical instillation (part B) of SCH 58500 (rAd/p53). Three patients were treated at each dose level, and dose escalation proceeded if no dose-limiting toxicity was observed. A dose-limiting toxicity was defined as any World Health Organization (WHO) grade 4 toxicity or any WHO grade 3 toxicity lasting more than 1 week. Adverse events that were clearly related to cystoscopy, catheter placement, cystectomy, or palliative treatment to the tumor were not considered dose-limiting. The protocol was approved by the local ethics committee (Bezirksärztekammer Rheinhessen) and the National Regulatory Office (Kommission Somatische Gentherapie der Bundesärztekammer). The study was conducted according to the Declaration of Helsinki (amended version, Hong Kong, 1989) and following the principles of good clinical practice.

Study Treatments

SCH 58500 is a replication-defective recombinant adenoviral vector encoding the complete human wild-type *p53* cDNA.[20,29] Doses were 7.5×10^{11} particles in level 1, 7.5×10^{12} particles in level 2, and 7.5×10^{13} particles in level 3. Patients treated in part A received a single intratumoral injection of 1 mL SCH 58500 in a standard saline-based solution[20] at cystoscopy on day 1. Patients treated in part B received a single intravesical instillation (total volume, 120 mL) of SCH 58500 in 20 mg/mL solution of Big CHAP, a transduction-enhancing agent,[28] through a transurethral catheter on day 1. After instillation, the catheters were blocked to allow a contact time of 60 minutes, followed by release of the catheter and extensive bladder irrigation with saline. During the course of the study, the vector instillation was divided into two sequential administrations of 50% of the vector dose each. The planned contact time for each half dose was 30 minutes; the second instillation immediately followed the release of the first dose. After treatment, all patients were hospitalized in single rooms in a biosafety environment at the study center for at least 24 hours or until adenovirus shedding was no longer detectable. Approximately 48 hours after vector administration (day 3), all patients underwent routinely scheduled radical cystectomies, which were not part of the study treatment.

Study End Points

The primary objective of this study was to assess the safety, feasibility, and toxicity of a single dose of SCH 58500 administered by intratumoral injection (part A) or by intravesical instillation (part B) in patients with invasive bladder cancer. Secondary end points were to

Table 1. Sequences of the Oligonucleotide Primers and Probes Used in Real-Time RT-PCR Assays

Target Gene	Function	Sequence	Expected PCR Product Size (bp)
p21/WAF1	Forward primer	TGGAGACTCTCAGGGTCGAAA	65
	Reverse primer	GGCGTTTGGAGTGGTAGAAATC	
	Probe	CGGCGGCAGACCAGCATGAC	
SCH 58500 DNA and RNA	Forward primer	AACGGTACTCCGCCACC	94
	Reverse primer	CGTGTCACCGTCGTGGA	
	Probe	CAGCTGCTCGAGAGGTTTTCCGATCC	
GAPDH	Forward primer	GAAGGTGAAGGTCGGAGTC	226
	Reverse primer	GAAGATGGTGATGGGATTTC	
	Probe	CAAGCTTCCCGTTCTCAGCC	

NOTE. All of the probes were labeled with the reporter signal FAM and TAMRA as the quencher.

assess vector distribution in normal and malignant bladder tissue, transgene expression, and markers of biologic activity in samples obtained at cystectomy.

Clinical Monitoring

Patients were closely monitored for adverse events for the first 7 days after study treatment. After hospital discharge, the patients were followed bimonthly for 1 year at the study center. The monitoring for the first 7 days after treatment included assessment of clinical symptoms, physical examination, monitoring of vital signs, Karnofsky index, concomitant medication, and recording of adverse events. Hematology, serum chemistry, and urinalysis were performed before treatment and on days 1, 2, 4, and 6 and during follow-up visits.

Virology Studies

Adenovirus shedding was monitored in urine, stool, or rectal swab specimens by means of a qualitative enzyme-linked immunosorbent assay (ELISA) before treatment on days 2 and 3 and until no adenovirus shedding was detectable.[20] In addition, urine samples were collected at multiple time points after study treatment and were examined for the presence of infectious adenoviruses by a flow cytometry–based infectivity assay.[30]

Detection of SCH 58500 DNA and Expression of Transgenic p53, p21/WAF1, and the Coxsackie and Adenovirus Receptors

SCH 58500 virus DNA, vector-specific transgene expression, p53 target gene *p21/WAF1* expression,[31,32] and Coxsackie and adenovirus receptor (CAR) expression were assessed in tumor samples and normal bladder tissue obtained at cystectomy by reverse transcriptase polymerase chain reaction (RT-PCR), as described previously,[20] and quantitative real-time PCR,[33,34] as described previously.[35] In brief, DNA and RNA were coextracted from frozen bladder samples using Trireagent (Molecular Research Center, Cincinnati, OH). Extracted RNA was DNased, and PCR was performed to ensure no DNA contamination. Real-time quantitative PCR and RT-PCR were performed using the ABI 7700 sequence detector (Applied Biosystems, Foster City, CA). The *GAPDH* gene was used as an internal control to assess the quality of assay samples. Gene expression results were expressed as number of copies per 1,000 copies of *GAPDH*. SCH 58500 DNA was quantified by comparison to viral DNA extracted from purified SCH 58500 virus (Qiagen, Valencia, CA). cRNAs were used as standards to quantify *p53*, *p21*, and *GAPDH* gene expression. The sequences of the oligonucleotide primers and probes are listed in Table 1. Primers for SCH 58500 gene and its expression were designed specifically to amplify SCH 58500 but not the human *p53* gene. Whenever possible, assays were performed on at least two different samples of tumor or nontumor tissue per patient. Bladder tissue samples obtained from patients with advanced bladder cancer, not treated with SCH 58500 served as negative controls. A cutoff level for positive real time PCR samples was set as the detection of at least 10 copies per reaction.[35]

Analysis of Tissue Sections

Localization of SCH 58500 was assessed using a direct in situ PCR method.[36] Formalin-fixed paraffin-embedded tissues were cut into 5-μm sections, placed on in situ PCR slides, and baked for 2 to 3 hours at 60°C on a slide hot plate. The slides were washed in xylene to remove the paraffin, followed by an incubation with 0.02 N HCl and digestion with 2.5 μg/mL proteinase K (Qiagen) at 37°C for 30 minutes. The endogenous alkaline phosphatase activity was eliminated by incubating the slides in ice-cold 20% (vol/vol) acetic acid. Slides were dehydrated in graded alcohols and rehydrated in 45 μL of PCR master mix containing 1 μmol/L of each dinitro-phenyl (DNP)-labeled primer, 200 μmol/L of each dNTP, 2.5 mmol/L magnesium chloride, and 10 units of AmpliTaq DNA polymerase (Applied Biosystems). Primers were designed to amplify a SCH 58500-specific sequence located between the cytomegalovirus promoter (5'-CGTGTCAC-CGTCGTGGA-3') and the upstream *p53* cDNA (5'-CCACTGCT-TACTGGCTTATCGAAAT-3'). This primer selection prevents the amplification of genomic *p53* DNA.[29] Reactions were performed in a Perkin Elmer Gene Amp In Situ PCR System 1000 (Applied Biosystems) programmed for one cycle of denaturation at 95°C for 5 minutes and annealing at 55°C for 90 seconds, followed by 34 cycles of 94°C for 30 seconds and 55°C for 90 seconds. After completion of the PCR, slides were washed two times with standard saline citrate (0.3 mol/L NaCl and 0.03 mol/L sodium citrate) and blocked with casein solution (Vector, Burlingame, CA). For tissue sections, the DNP molecules incorporated into the PCR amplicons were detected using an anti-DNP antibody conjugated with alkaline phosphatase (Applied Biosystems). The sections were stained using the alkaline phosphatase substrate NBT/BCIP (nitro-blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate) (Boehringer Mannheim, Germany) and then counterstained with Nuclear Fast Red (Vector). As a negative control, each section was processed, but the PCR reaction was performed without AmpliTaqDNA polymerase. Samples from rat bladders instilled with SCH 58500 or a *β-galactosidase*–expressing adenoviral vector (Ad5.β-gal) served as positive and negative controls.

Table 2. Patient Demographics, Tumor Stages, Histologies, and Transgene Expression After SCH 58500 Treatment

Patient No.	Age (years)	Sex	Histology	p53	Stage	Study Group	RT-PCR
001	68	Male	TCC	0	pT4aN2M0 G4	A1	–
002	37	Female	SCC	2	pT3bN0M0 G2-3	A1	–
003	64	Male	TCC	1	pTaN0M0 G2-3	A1	–
004	69	Male	TCC	3	pT1N0M0 G2	B1	+
005	69	Male	TCC	1	pT3aN0M0 G2	B1	+
006	73	Male	TCC	0	pT3aN0M0 G2	B1	+
007	69	Male	TCC	1	pTisN0M0 G3	B2	+
008	69	Male	SCC	3	pT3aN0M0	B2	–
009	70	Male	TCC	0	pT1N0M0 G3	B2	+
010	60	Female	TCC	1	pT1N1M1	B3	ND
011	84	Male	TCC	1	pT2bN1M0 G3	B3	+
012	82	Male	TCC	2	pT4N0M0 G3	B3	+

Abbreviations: TCC, transitional cell carcinoma; SCC, squamous cell carcinoma; Stage, tumor stage according to tumor-node-metastasis classification; p53, immunohistochemical detection of nuclear p53 expression in baseline tumor biopsies in < 10% of tumor cells = 0, 11%-25% of tumor cells = 1, 26%-50% of tumor cells = 2, > 50% of tumor cells = 3; A1, intratumoral injection (part A), dose level 1 (7.5 × 10^{11} particles); B1, intravesical instillation (part B), dose level 1 (7.5 × 10^{11} particles); B2, intravesical instillation, dose level 2 (7.5 × 10^{12} particles); B3, intravesical instillation, dose level 3 (7.5 × 10^{13} particles); RT-PCR, positive (+) or negative (–) expression of vector-specific *p53* RNA as detected by RT-PCR analysis of samples obtained at cystectomy; ND, not determined (no cystectomy performed).

The protein expression of p53, *p21/WAF1*, apoptosis-related and cell cycle–related genes, and CAR was assessed by immunohistochemistry in formalin-fixed paraffin-embedded tissue sections. Primary antibodies against p53 (M7001, Dako Diagnostika, Hamburg, Germany), *p21/WAF1* (M7202, Dako), Bcl-2 (M0887, Dako), Bak (AM04, Calbiochem, San Diego, CA), Bax (Ab-1/PC66, Calbiochem), MIB1 (dia 505, Dianova, Hamburg, Germany), and CAR (a gift from Dr Robert W. Finberg, Dana-Farber Cancer Institute, Boston, MA[37,38]) were used. Apoptotic cells were visualized by microscopy following the terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate-biotin nick end-labeling (TUNEL) method[39] and by means of laser scanning cytometry, as previously described.[40] Normal bladder and tumor tissue samples from patients not treated with SCH 58500 served as controls.

RESULTS

Enrollment and Treatments

Twelve patients from a single center were enrolled onto the study. Baseline characteristics and histologies of the study patients are listed in Table 2. Three patients were treated at dose level 1 in part A (intratumoral injection) of the study. No additional dose escalation was performed in part A. Nine patients were treated at three different dose levels in part B (intravesical instillation). Eleven patients underwent radical cystectomies after study treatment. In one patient, the tumor was determined to be unresectable with curative intent at laparotomy. Thus, tumor samples for assessment of the secondary end points were obtained from 11 patients treated with SCH 58500 at three dose levels.

Toxicity

Postoperatively, one patient treated in part A suffered from WHO grade 1 fatigue. No toxicities were observed in the other two patients treated with intratumoral injection at cystoscopy. The predominant toxicities observed in patients treated in part B of the study were urethral and vesical burning, which reached WHO grade 2 in two patients and WHO grade 3 in another two patients. In addition, one patient each experienced WHO grade 2 and grade 3 abdominal pain. These symptoms were relieved in two patients treated at dose level 1 by a reduction of the contact time, for which the transurethral catheters were clamped. Hence, for patients treated at dose levels 2 and 3, the treatment was administered in two sequential 30-minute sessions. Additionally, patients treated at dose level 3 were premedicated with 50 mg of pethidine and 20 mg of butylscopolamine. Despite these modifications, the planned contact time had to be reduced by several minutes in three patients treated at dose level 2 and in one patient treated at dose level 3. All symptoms resolved immediately after release of the transurethral catheter and bladder irrigation with saline. No fever, chills, or other signs of systemic toxicity were observed in patients treated in part B. No alterations of laboratory parameters, including liver enzymes and bilirubin, were detected before surgery on day 3. Three patients were hospitalized because of fever of unknown origin within 4 to 6 weeks after surgery and quickly recovered under treatment with broad-spectrum antibiotics. In one of these patients, a methicillin-resistant *Staphylococcus aureus* was isolated from a catheter. Thus, even at the highest dose level of 7.5 × 10^{13} particles SCH 58500 administered by intravesical instillation, no dose-limiting toxicities were observed.

Transgene Expression and Biologic Activity

In two of three assessable patients treated in part A (intratumoral injection), vector DNA was found by PCR

Table 3. Induction of p21/WAF1 RNA and Protein Expression After Intravesical SCH 58500 Treatment

Patient No.	Study Group	Normal Bladder Tissue		Tumor Tissue	
		p21 RNA	p21 IHC	p21 RNA	p21 IHC
Controls	—	1.08 ± 1.8	—	0.26 ± 0.38	—
004	B1	3.5 ± 2.2	0/0	0.92 ± 1.12	0/2
005	B1	2.47	0/0	1.16 ± 1.37	0/2
006	B1	.57 ± 0.4	0/0	1.16 ± 1.43	0/0
007	B2	2.62	0/0	0.32 ± 0.29	0/0
008	B2	ND	0/0	0.32 ± 0.29	0/0
009	B2	4.59	0/0	0.61	0/0
010	B3	ND	ND	ND	ND
011	B3	1.29 ± .42	0/0	10.4 ± 20.69	0/2
012	B3	2.66 ± 1.56	0/0	3.99 ± 4.7	0/1

Abbreviation: ND denotes not determined (insufficient sampling or no cystectomy performed).

NOTE. Tissue samples from bladder tumors and normal bladder tissue obtained at cystectomy were examined by real-time RT-PCR (p21 RNA) and immunohistochemistry (p21 IHC). Normal bladder samples from four patients and tumor samples from five patients not treated with SCH 58500 served as controls for real-time RT-PCR (Controls). Results from real-time RT-PCR are expressed as mean ± SD × 10,000 copies normalized to 1,000 copies *GAPDH* RNA. Results from immunohistochemistry are presented as nuclear expression of p21/WAF1 in biopsies before and after SCH 58500 treatment (< 10% of tumor cells = 0; 11%-25% of tumor cells = 1; 26%-50% of tumor cells = 2; > 50% of tumor cells = 3).

analysis of posttreatment tumor samples (not shown). However, no transgene expression as assessed by RT-PCR analysis of vector-specific *p53* expression was detected after intratumoral injection of SCH 58500 at cystoscopy (Table 2). In contrast, vector-specific *p53* transgene expression was found by RT-PCR analyses of tissue samples from seven of eight assessable patients treated with intravesical instillation of SCH 58500 (Table 2).

To address whether the *p53* transgene expression translated into biologic activity, we determined the quantitative expression of the p53 target gene *p21/WAF1* by real-time RT-PCR analysis of tumor and normal bladder samples from patients treated with intravesical instillation of SCH 58500 or untreated control patients. The *p21/WAF1* expression in tumor samples from untreated control patients was lower than in normal bladder samples (Table 3). Assaying nontumor bladder samples from patients treated with SCH 58500 instillation, moderate changes in *p21/WAF1* expression were detected when compared with untreated controls (Table 3). However, in tumor samples from patients treated at the highest dose level of 7.5×10^{13} particles SCH 58500 *p21/WAF1*, expression was increased up to 40-fold compared with control tumor samples from patients not receiving gene therapy (Table 3). Immunohistochemical analyses revealed an increased *p21/WAF1* protein expression after SCH 58500 treatment in tumor tissues but not in normal bladder samples from four patients with undetectable or low *p21/WAF1* protein expression at baseline (Table 3). No significant correlation between transgene expression, *p21/WAF1* induction, and CAR expression, as determined by RT-PCR analysis and immunohistochemistry, could be established. However, the CAR expression detected by immunohistochemistry exhibited a considerable heterogeneity among tumors from different patients as well as among different regions of the same tumor (not shown). Immunohistochemical analyses of p53 expression or expression of additional apoptosis-related or cell cycle–related genes revealed no consistent changes in relation to SCH 58500 treatment. Moreover, we failed to detect a significant induction of apoptosis as assessed by TUNEL staining and microscopy or laser scanning microscopy in samples taken at cystectomy approximately 48 hours after SCH 58500 treatment (not shown).

Taken together, these data demonstrate that a detectable p53 transgene expression in bladder tumors can be achieved by intravesical instillation of SCH 58500 in combination with a transduction-enhancing agent. At the highest dose level of 7.5×10^{13} particles SCH 58500, evidence for biologic activity in terms of RNA and protein expression of the p53 target gene *p21/WAF1* was obtained.

Vector Distribution

Using quantitative real-time PCR, SCH 58500 DNA copies were detected in normal bladder and tumor samples from patients treated with intravesical instillation in a dose-dependent manner, whereas no SCH 58500 DNA was found in samples from control patients not treated with SCH 58500 (Fig 1). The demonstration of vector DNA or transgene expression in tissue homogenates does not provide information regarding the transduction efficacy or the vector penetration. Therefore, tissue sections from patients treated in part B were analyzed by in situ PCR, revealing a strong vector-specific signal throughout the whole urothelium (Fig 2). Moreover, SCH 58500 DNA was also detected

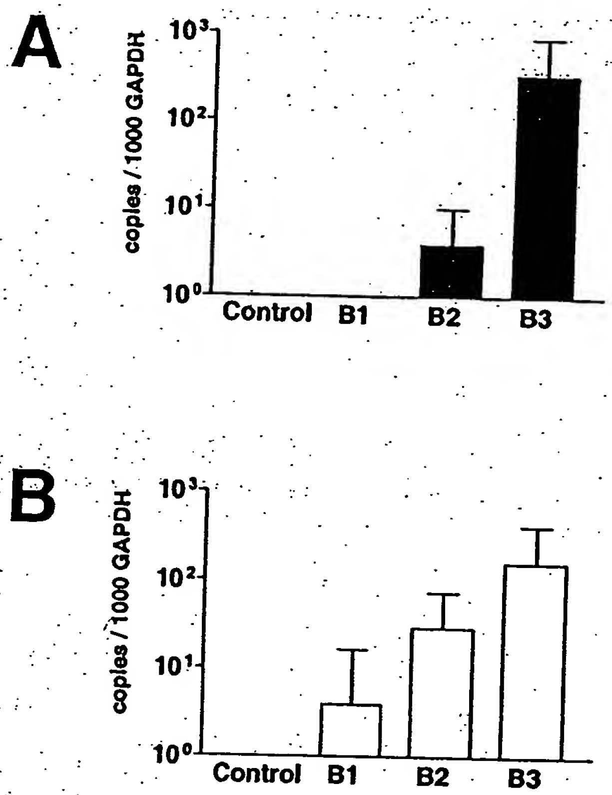

Fig 1. Quantitative detection of SCH 58500-specific DNA sequences (mean ± SD) by real-time PCR analysis of samples from tumor (A) and nontumor bladder tissue (B) of untreated bladder cancer patients (control) and patients treated with intravesical instillation of SCH 58500 at dose levels 1 (B1), 2 (B2), and 3 (B3).

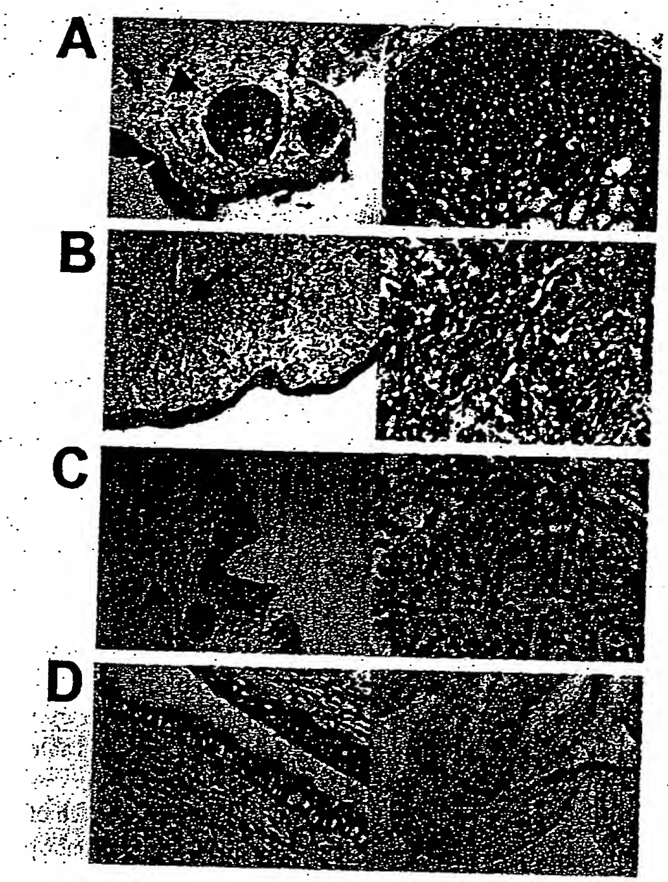

Fig 2. SCH 58500 vector distribution (in situ PCR) in tissue sections from patient no. 011 (A and B) and patient no. 005 (C) treated with intravesical instillation. Arrowheads indicate submucosal tumor nodules (A and C); arrow indicates cells in the Lamina propria (B). Sections from rat bladders injected with SCH 58500 (D, left panel) or control virus (D, right panel) are shown as positive and negative controls.

in submucosal tumor nodules as well as in cells in the Lamina propria. Thus, intravesical instillation of SCH 58500 in combination with a transduction-enhancing agent can achieve an uniform vector penetration throughout the urothelium as well as into submucosal tumor tissues.

Virologic Studies

After SCH 58500 treatment, all patients in both study groups underwent extensive bladder irrigation with 6 L saline through a transurethral catheter over a period of 36 to 48 hours. Excretion of infectious adenoviruses was detected by a sensitive flow cytometry-based assay[30] in samples taken from the first 2 to 4 L of void volume. No detectable urinary adenovirus excretion was found after 6 L of bladder irrigation (Fig 3). None of the urine samples taken 24 hours after study treatment gave a positive result in the qualitative on-site ELISA assay (not shown).

Long-Term Follow Up

Nine of the 12 study patients were alive at a median follow-up of 30 months. In addition to SCH 58500 treatment and radical cystectomy, two patients received a platinum-based adjuvant chemotherapy regimen. In one patient treated in part A, fulminant liver metastases developed 4 weeks after surgery, which were not detectable on computed tomogram and ultrasound examinations performed at the preoperative staging. The patient was treated with palliative chemotherapy, but he died from progressive liver failure 7 weeks after cystectomy. One patient treated in part A developed a *Mycoplasma* pneumonia during adjuvant chemotherapy. In total, three patients died from disease progression, and one patient is being treated with palliative chemotherapy for recurrent disease.

DISCUSSION

A major challenge in the conservative management of localized bladder cancer is the frequent recurrence and progression to an advanced tumor stage in patients with high-risk tumors.[2] To improve disease control, local tumor

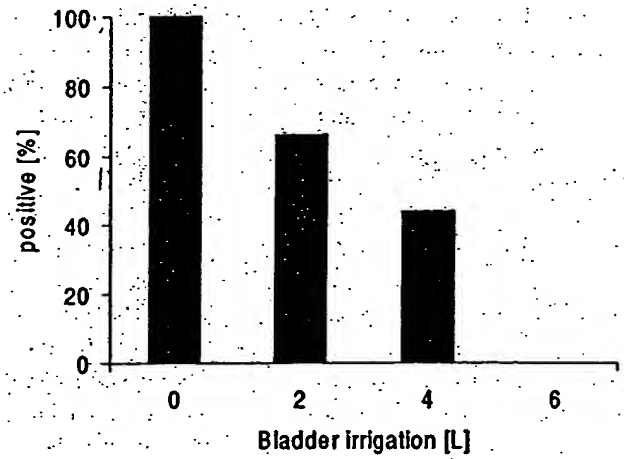

Fig 3. Excretion of infectious adenoviruses after intravesical SCH 58500 treatment. The percentage of patients (n = 12) with urine samples positive for infectious adenoviruses after the indicated volumes of bladder irrigation with saline.

resection is combined with intravesical therapy with bacillus Calmette-Guerin or anticancer agents.[8] However, these treatments have a substantial toxicity and may reduce the risk of recurrences but do not prevent disease progression.[6,7] Thus, new treatment options for high-risk superficial bladder cancer are required. Mutations of the *p53* tumor suppressor gene are frequently found in bladder cancer, are associated with an adverse prognosis in some studies, and may contribute to a more aggressive clinical course and resistance to anticancer treatment.[14,15] In an ortopic injection model of bladder cancer, *p53* gene transfer acted synergistically with cisplatin to prevent tumor growth and induce apoptosis in vivo.[41]

In the present phase I study, we tested whether adenoviral vector-mediated wild-type *p53* gene transfer is safe, feasible, and biologically active in patients with invasive bladder cancer. Taking advantage of the anatomy of the bladder, we planned to evaluate two different modes of vector administration: the intratumoral injection of vector solution, because it has been performed in several clinical studies of cancer gene therapy,[19-21] and the intravesical vector instillation via a transurethral catheter. Because preclinical studies convincingly demonstrated that the transduction efficacy of adenoviral vectors instilled into the bladder can be dramatically enhanced by the addition of several compounds,[27,28] here we administered intravesical SCH 58500 in combination with the transduction-enhancing agent Big CHAP.[28] Both modes of administration of the study treatment, intratumoral injection at cystoscopy and transurethral intravesical instillation, were well tolerated and devoid of any detectable systemic toxicity. Successful gene transfer after intravesical instillation of SCH 58500 in combination with Big CHAP was detected by RT-PCR analysis in seven of eight assessable patients. Moreover, evidence for biologic activity of the transgene, as determined by quantitative RT-PCR analysis of RNA expression as well as by immunohistochemical analysis of protein expression of the p53 target gene *p21/WAF1*,[32] was found in patients treated at higher dose levels. Transgene expression did not seem to correlate with the CAR expression status of the tumor samples as determined by RT-PCR analysis and immunohistochemistry. However, the relatively small number of patients enrolled onto this study and the detection methods for CAR expression might have influenced this result. Compared with the effective transduction achieved by intravesical vector instillation, no evidence for transgene expression was detected in the three patients treated by intratumoral injection of SCH 58500 at dose level 1, whereas SCH 58500 DNA sequences were detectable in two patients by PCR analysis. This was surprising, given that in a previous study in patients with NSCLC treated by intratumoral injection of SCH 58500, *p53* transgene expression was detected in four of five assessable patients receiving the same vector dose of 7.5×10^{11} particles.[20] Because intratumoral vector injection at cystoscopy is a relatively invasive procedure compared with transurethral vector instillation, it was decided not to proceed with the dose escalation in part A of this trial. Hence, we cannot exclude that at higher dose levels, a *p53* transgene expression in bladder tumors would have been achieved by intratumoral injection of SCH 58500 at cystoscopy. Furthermore, the addition of Big CHAP or other transduction-enhancing agents[28] might also be beneficial in the case of intratumoral vector injection in the bladder. However, in the light of the efficacy and ease of the intravesical instillation approach, intratumoral vector injection at cystoscopy clearly is the inferior approach for vector administration in bladder cancer.

In contrast to the results obtained with the p53 target gene *p21/WAF1*, we found no consistent changes in the expression of *p53*, various cell cycle–related or apoptosis-related genes, or TUNEL staining in response to SCH 58500 administration. This observation might be limited by the small number of patients enrolled onto the trial and the availability of only a single time point for these examinations. Moreover, the activity of many genes regulating apoptosis is not controlled by their expression level but by conformational changes or changes in their subcellular localization,[42] which cannot be detected by the methods applied in this study. Nevertheless, the *p21/WAF1* response is a valid marker for biologic activity of transgenic p53,

which has been confirmed in additional settings of clinical p53 gene therapy.[35,43]

In addition to molecular and immunohistochemical evidence for transgene expression and biologic activity, important information related to the vector distribution throughout the bladder and vector penetration into tumor tissues was gathered from this trial. We demonstrated by quantitative PCR analysis that administration of higher particle doses resulted in the recovery of higher copy numbers of SCH 58500-specific DNA from tissue samples (Fig 3). This was not unexpected; however, it suggests that together with the evidence for increased *p21/WAF1* expression at high doses, a plateau of the biologic activity was not reached by the intravesical instillation of 7.5×10^{13} particles SCH 58500. Presently, technical limitations preclude the administration of a more concentrated adenovirus solution, leaving this issue unresolved. With respect to the vector distribution after intravesical instillation, we found a uniform distribution of SCH 58500 DNA throughout the normal urothelium and the luminal tumor tissues by in situ PCR analysis of bladder sections. Moreover, vector DNA could also be found in apparently submucosal tumor nodules as well as in cells in the Lamina propria. These results confirm the hypothesis that the instillation approach results in an improved vector distribution. In addition, they demonstrate that even submucosal tumor cells can be targeted by the luminal administration of an adenovirus in combination with a transduction-enhancing agent in the bladder.

The optimal dosing schedule for intravesical SCH 58500 instillation remains to be established. Because of the procedure-associated discomfort observed in most patients treated in part B of this study, the contact times varied considerably. Yet SCH 58500 penetration and transgene expression analyses yielded promising results. It seems likely that even shorter contact times than the ones allowed in the course of this trial might result in sufficient transduction rates with lower local toxicity, a hypothesis supported by initial data from preclinical in vivo models. The intravesical instillation of SCH 58500 through a transurethral catheter also is environmentally safe, because infectious adenoviruses excreted in the urine after SCH 58500 treatment can easily be recovered in a contained system. The virologic studies performed in this trial suggest that if the bladder is sufficiently irrigated, infectious viral particles are only excreted with the first 4 L of irrigation fluid. This could minimize hospitalizations and could even allow outpatient treatment.

The design of the present phase I study precluded the collection of data regarding the long-term effects of the intravesical administration of such high-vector doses as well as signs for clinical efficacy. However, important and unique data demonstrating effective vector distribution, transgene expression, and biologic activity after a clinically practicable and safe gene transfer procedure were obtained in patients with invasive bladder cancer. These results provide a strong rationale for future investigation of adenovirus therapy in bladder cancer and support trials addressing the clinical efficacy of intravesical SCH 58500 treatment in patients with superficial high-risk bladder cancer.

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